RES79-EN.01

resDetect™ Gentamicin ELISA Kit (high sensitivity)

Pack Size: 96 tests

Catalog Number：RES-A079

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

INTENDED USE

The Gentamicin ELISA Kit was developed for the detection and quantitative determination of gentamicin residues in plasmid DNA raw materials and proteins for CGT, vaccine and other biological drugs. The kit is calibrated with NIFDC and USP standards, it's intended for research use only (RUO).

BACKGROUND

Residues of gentamicin are prone to occur in the production process of biological products, which can easily lead to abnormal reactions in the human body. Therefore, the residual amount of gentamicin in biological products or semi-finished products of biological products should be strictly controlled.

PRINCIPLE OF THE ASSAY

The Gentamicin ELISA Kit adopts the competitive ELISA method, and the pre-coated conjugated Gentamicin antigen on the microstrip competes with the residual Gentamicin in the sample to bind the enzyme-labeled anti-Gentamicin monoclonal antibody, and then uses a microplate reader to detect the absorbance value by adding TMB substrate, and the absorbance value is negatively correlated with the content of kanamycin in the sample. The kit only takes about one hour and 20 minutes to operate and has a linear range of 0.1 ng/mL to 3.2 ng/mL.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for kanamycin residue detection in CGT, vaccine and other biological drugs plasmid DNA and protein stocks.
3. For the detection of other biologics samples, user suitability verification is recommended to exclude dryness of the matrix interference.
4. Do not use reagents past their expiration date.
5. Do not mix or substitute reagents with those from other kits or other lot number kits.
6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.
7. If samples generate values higher than the highest standard, dilute the samples with the Dilution

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Buffer provided in kit and repeat the assay.

8. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials Provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RES-A025-C01	Gentamicin Coated Plate	1 plate	Solid	2-8°C	2-8°C
RES-A025-C02	Gentamicin Standard	0.1152 μg	Power	2-8°C	-70°C
RES-A025-C03	HRP-Anti-Gentamicin	6 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES-A025-C04	1×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES-A025-C05	20×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES-A025-C06	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES-A025-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

SRORAGE

1. Unopened kit should be stored at 2°C -8°C upon receiving.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.
3. The reconstructed Gentamicin standard is stored at -70°C in at least 300 μL per tube and cannot be frozen and thawed repeatedly.

Note: *a. Do not use reagents past their expiration date.*

b. Find the expiration date on the outside packaging.

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REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection requirements;

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Tubes;

Timer;

Reagent bottle;

Deionized or distilled water.

NOTICE BEFORE MEASUREMENT

1. Bring all reagents and samples to room temperature (20℃-25℃) before use.
2. Immediately return all reagents to 4℃ after use.
3. The plates can be opened only after all samples have been prepared, and the unused plates are immediately returned to the sealed bag provided with the kit and stored away from light.
4. According to Table 2, prepare the Gentamicin standard into a storage solution with ultrapure water, dissolve at room temperature for 10 minutes, and shake gently and mix well. The reconstructed kanamycin standard is stored at -70℃ in at least 300 μL per tube and cannot be frozen and thawed repeatedly.

Table 2. Preparation method

ID	Components	Size (96 test)	Storage solution concentration.	Reconstituted water Vol.
RES-A079-C02	Gentamicin Standard	0.1152 μg	64 ng/mL	1.8 mL

Recommended Sample Preparation

Working Solution Preparation

1. Preparation of 1×Washing Buffer:

Dilute 25 mL 20×Washing Buffer with ultrapure water/deionized water to 500 mL.

2. Sample preparation:

Most samples are diluted according to the dilution ratio confirmed by the interference of the samples themselves.

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RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

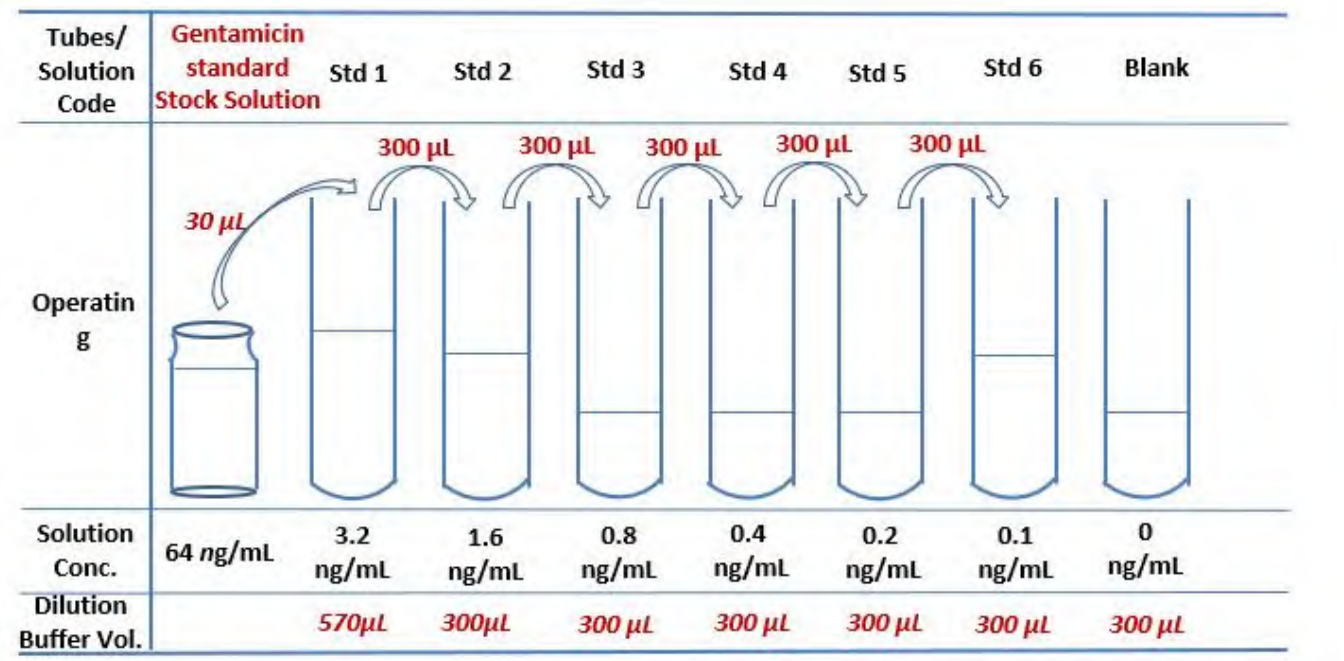
Dilute 25 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Sample preparation

Most samples are diluted according to the dilution ratio confirmed by the interference of the samples themselves.

2. Preparation of Standard curve

The concentration of the reconstituted Gentamicin Standard (RES079-C02) is 64 ng/mL， prepare (Std1) by diluting 30 µL the reconstituted Gentamicin Standard into 570 µL 1×Dilution Buffer mix gently well. Then Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 µL of Sample Dilution Buffer into each tube. Make sure to mix well every time. 1×Dilution Buffer serves as (Blank) 0 ng/mL.



3. Add Samples and Antibody

Add 50 µL samples to each well. For Blank Control wells, please add 50 µL Dilution Buffer. Then add 50 µL HRP-Anti-Gentamicin,.

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***Note**: It is recommended to set double holes for samples and standard curves to be tested.*

4. Incubation

Seal the plate with microplate sealing film and incubate at room temperature (20℃-25℃) for 1 hour, avoid light.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 30 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (20℃-25℃)for 20 min, avoid light.

7. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

***Note:** The color in the wells should change from blue to yellow.*

8. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

***Note**: To reduce the background noise, subtract the value read at OD_{450nm} with the value read at $OD_{630\ nm}$.*

Calculation of Result

1. The standard curve is plotted with the standard concentration as x-axis and Log the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
2. Normal range of Standard curve: $R^2 \geq 0.9900$.
3. Detection range: 0.1 ng/mL-3.2 ng/mL. If the OD value of the sample to be tested is higher than 3.2 ng/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 0.1 ng/mL, the sample should be reported.

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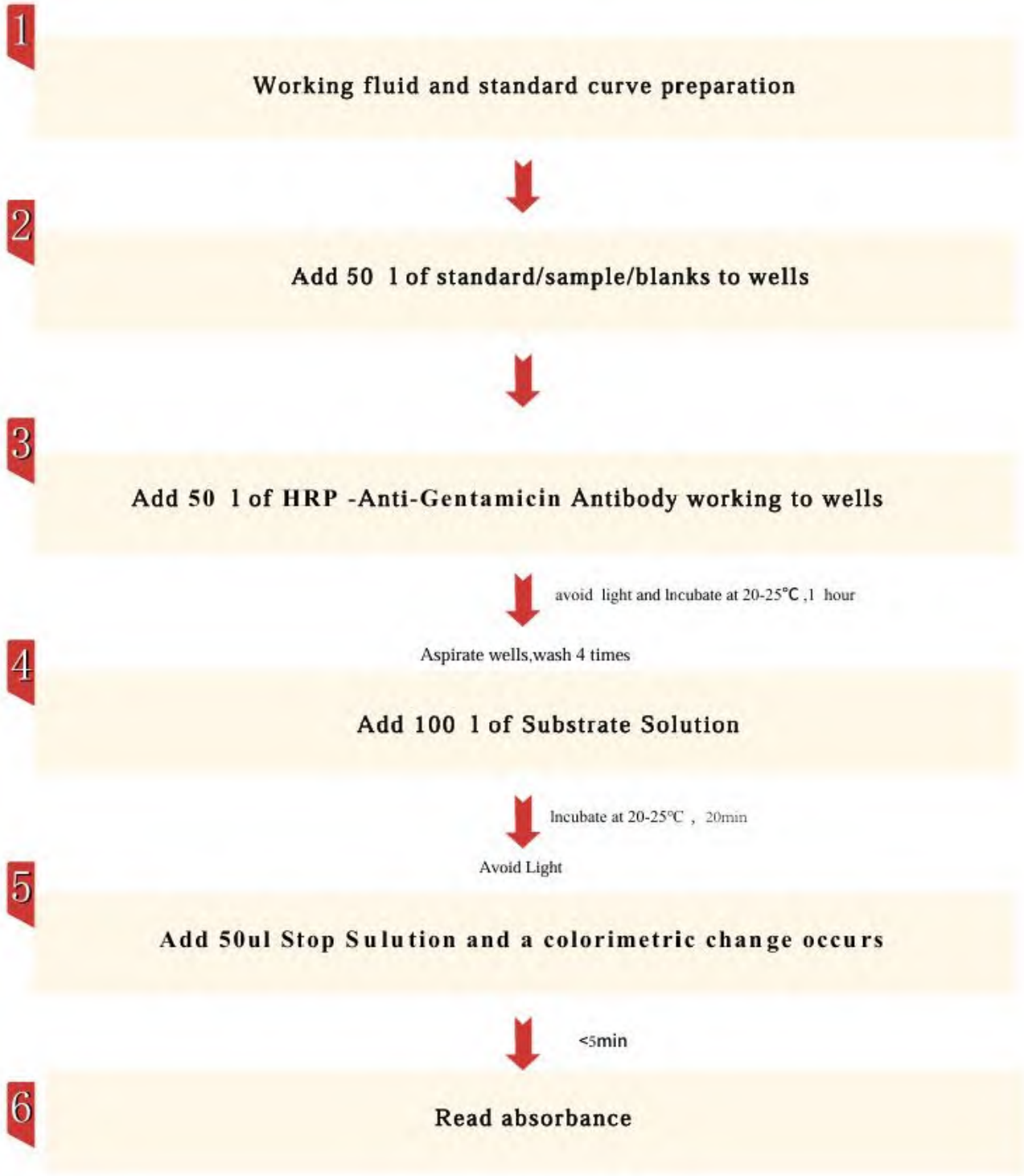
QUICK GUILD



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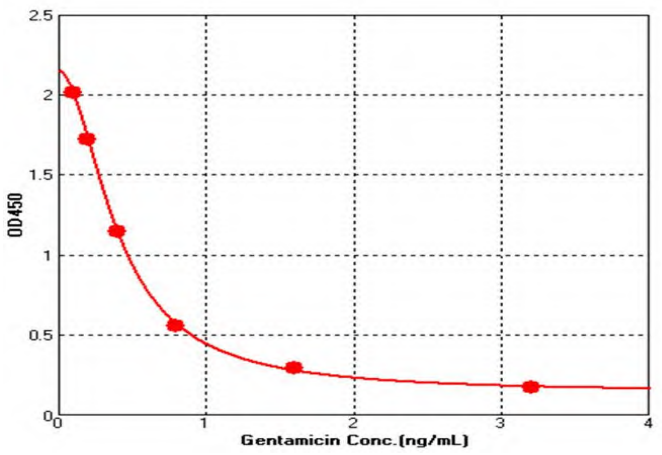
Typical Data

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (ng/mL)	OD450-1	OD450-2	Average
3.2	0.172	0.170	0.171
1.6	0.282	0.301	0.292
0.8	0.542	0.579	0.561
0.4	1.126	1.165	1.146
0.2	1.713	1.731	1.722
0.1	1.999	2.022	2.011



Limit of Quantitation

The linear interval was 0.1 -3.2 ng/mL, R2 > 0.99, When the concentration recovery rate was between 80-120% and OD value CV ≤ 20%, the maximum concentration corresponding to 3.2 ng/mL was confirmed as the upper limit of quantification of the kit (ULOQ). When the concentration recovery rate was between 75%-120% and OD value CV≤20%, the corresponding minimum concentration was 0.1 ng/mL, which was confirmed as the lower limit of quantitation (LLOQ) of the kit.

/	Upper limit of quantitation (ULOQ) (3.2 ng/mL)	Lower limit of quantitation (LLOQ) (0.1 ng/mL)
OD CV (%)	5	6
Recovery Rate (%)	93	120

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision,and the detection concentration CV⩽15%.

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2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision, and the detection concentration CV≤15%.

	Intra-assay Precision			Inter-assay Precision		
Sample Conc.(ng/mL)	3.2	0.5	0.1	3.2	0.5	0.1
n	10	10	10	10	10	10
Mean (ng/mL)	2.974	0.497	0.120	2.957	0.466	0.117
SD	0.041	0.134	0.122	0.328	0.467	0.017
CV%	14	9	5	12	6	15

ACCURACY

Five samples of different concentration were tested ten times , and the range of the recovery rate were 75%-120%.

Samples	1	2	3	4	5
Sample Conc.(ng/mL)	3.2	2.4	0.5	0.25	0.1
n	10	10	10	10	10
Mean (ng/mL)	2.974	2.605	0.497	0.299	0.120
SD	0.041	0.035	0.134	0.204	0.122
CV(%)	14	11	9	9	5
Recovery Rate (%)	93	109	99	92	120

SPECIFICITY

1. Cross-reactivity

When 500 μg/mL ampicillin, tetracycline and chloramphenicol were added into the sample diluent, no cross-reactivity was observed.

Cross Reactant	Cross-reactivity
Gentamicin (500 μg/mL)	100%
Ampicillin (500 μg/mL)	＜1%

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Tetracycline (500 μg/mL)	＜1%
Chloramphenicol (500 μg/mL)	＜1%

2. Interference

When 2000 ng/mL of E.coli HCP, 500 ng/mL of E.coli HCD and 100 ng/uL of plasmid DNA were added into the diluent, the recovery rates of the three samples of known concentration were 75%-125%.

Cross Reactant	E.coli HCP Conc. (2000 ng/mL)			E.coli HCD Conc. (200 ng/mL)			Plasmid DNA Conc. (200 ng/uL)		
Sample Conc.(ng/mL)	3.2	0.5	0.25	3.2	0.5	0.25	3.2	0.5	0.25
Detected Sample Conc. (ng/mL)	3.050	0.556	0.298	3.196	0.455	0.277	3.934	0.543	0.217
Recovery Rate (%)	95	111	119	100	91	111	123	109	87

INTERFERING SUBSTANCES

We have conducted interference effect test about frequently-used buffers, adding the known concentration of Gentamicin standard into the buffer, and the calculated recovery rate was 75%-120%, they have excellent buffer compatibility. For specific buffers, it is recommended that you verify recovery to determine the optimal dilution ratio.

	Gentamicin Standard	
Matrix	Recovery %	Dilution Factor
20 mM L-histidine with 0.1% (w/v) PF68, pH6.0	99	2
20 mM L-histidine with 0.4% (w/v) Tween-80, pH6.0	103	1
1×PBS, pH7.3	94	1
1*PBS, pH7.3 with 11% Trehalose	98	1
20 mM L-histidine, pH6.0	79	2
50 mM Tris,100 mM Glycine, pH7.5	111	2
100 mM Tris,20 mM Sodium citrate, pH7.5	114	2
20 mM L-histidine 10% trehalose,pH6.0	115	1
25 mM Phosphate, pH 7.5	75	2
25 mM Phosphate, pH 7.5	90	2

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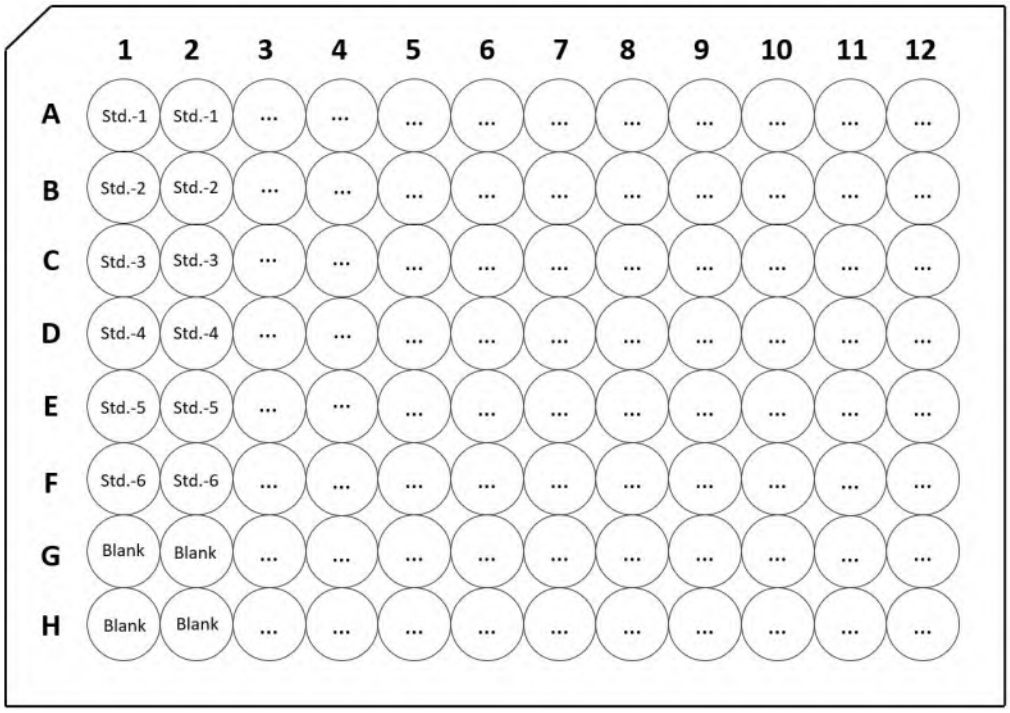
100 mM Glycine, pH 3.5	108	1
100 mM Triscitrate, 7.5	105	1

PLATE LAYOUT



TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time

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Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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